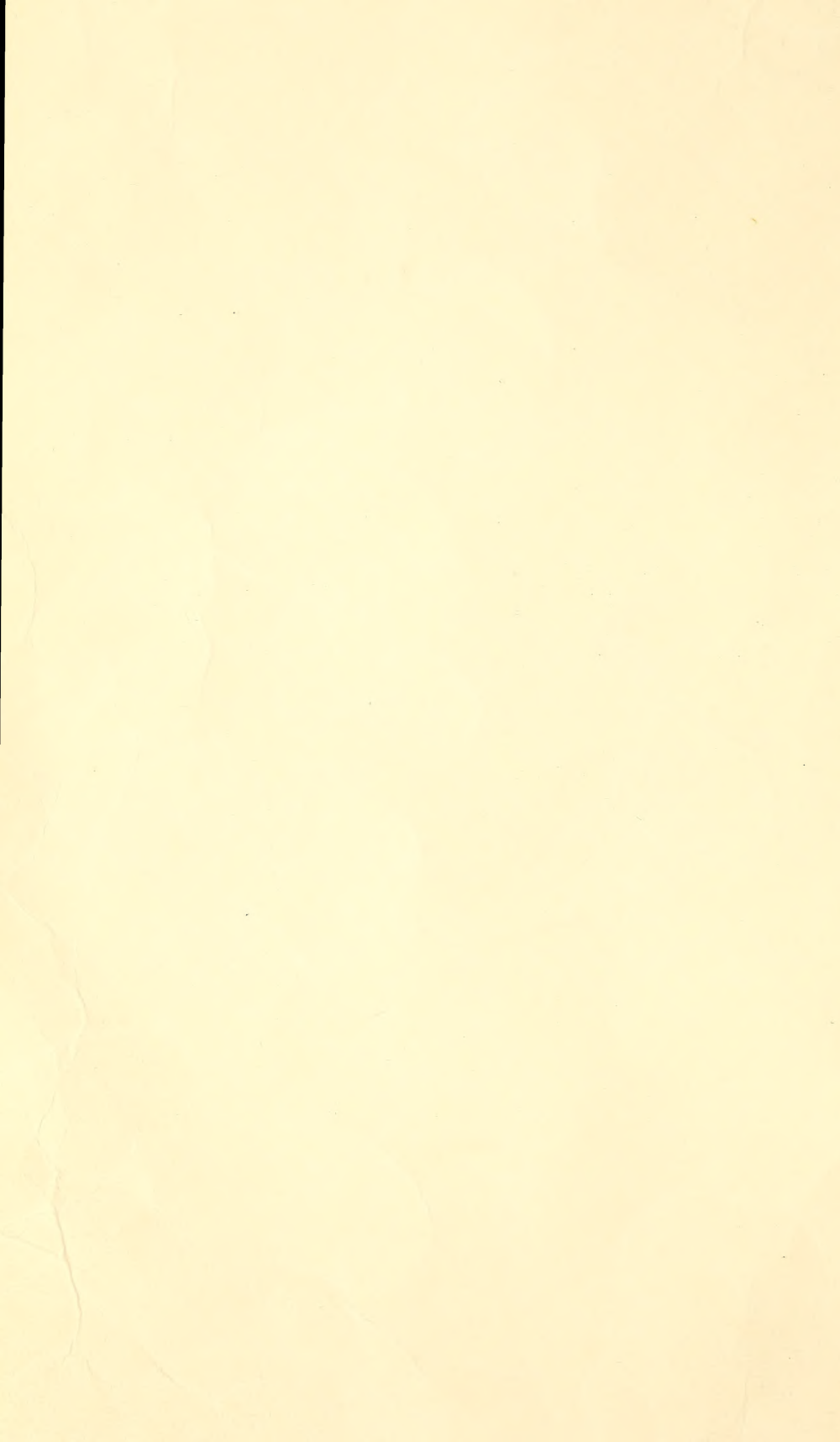






# Historic, archived document

Do not assume content reflects current scientific knowledge, policies, or practices.



## UNITED STATES DEPARTMENT OF AGRICULTURE



## BULLETIN No. 454

Contribution from the Bureau of Plant Industry  
WM. A. TAYLOR, Chief



Washington, D. C.

PROFESSIONAL PAPER

December 9, 1916

## THE EFFECT OF CULTURAL AND CLIMATIC CONDITIONS ON THE YIELD AND QUALITY OF PEPPERMINT OIL.

By FRANK RABAK,

*Chemical Biologist, Drug-Plant and Poisonous-Plant Investigations.*

## CONTENTS.

|                                                                              | Page. |                                                                                                                    | Page. |
|------------------------------------------------------------------------------|-------|--------------------------------------------------------------------------------------------------------------------|-------|
| Introduction.....                                                            | 1     | Physical and chemical properties of peppermint oil from fresh and from dry plants at various stages of growth..... | 5     |
| Effect of soil and climate on the composition of peppermint oil.....         | 2     | Effect of light and shade.....                                                                                     | 11    |
| Yield of oil from fresh and from dry plants at various stages of growth..... | 3     | Effect of frost action.....                                                                                        | 12    |
|                                                                              |       | Conclusions.....                                                                                                   | 15    |

## INTRODUCTION.

The peppermint plant, known botanically as *Mentha piperita* L., is cultivated extensively both in the United States and foreign countries as the source of a volatile oil which is widely used as a flavoring and therapeutic agent. The value of the oil depends much upon its composition. The principal ester constituent, menthyl acetate, possesses a very fragrant minty odor, to which the agreeable aroma of the oil is largely due. The alcoholic constituent, menthol, possesses the well-known penetrating minty odor and characteristic cooling taste. The flavoring properties of the oil are due largely to both the ester and alcoholic constituents, while the medicinal value is attributed to the latter only. The elaboration of these constituents depends upon the various cultural and climatic conditions to which the plant is subjected during growth.

In order to obtain data bearing on possible variations in the composition of peppermint oil under varying conditions, plants were grown under various conditions of soil and climate and were harvested at various stages of growth. The oil was distilled from certain parts and also from the entire herb in both the fresh and the dry condition and was then subjected to thorough examination.

## EFFECT OF SOIL AND CLIMATE ON THE COMPOSITION OF PEPPERMINT OIL.

Henderson states <sup>1</sup> that peppermint plants grown on a river bank on heavy loam and on deep sandy loam yielded oils which showed distinct differences in composition. According to Umney,<sup>2</sup> soil and climatic conditions are responsible for the higher content of esters in some peppermint oils than in others. Charabot and Hébert<sup>3</sup> have shown that the formation of certain constituents of the oil, and especially the esters, is favored by treating the soil with sodium chlorid and sodium nitrate. Mossler<sup>4</sup> has also observed that certain fertilizers, such as saltpeter, superphosphate, and potash, tend to favor the growth of the plant and the production of oil therein.

In order to study the effect of various types of soil and climatic conditions upon the general composition of peppermint\*oil, plants were grown at Webster, S. Dak., on rich black loam soil; at Glenn Dale, Md., on light sandy soil; and at Arlington Farm, Va., on heavy clay soil. The yields of oil distilled from these plants and also the physical and chemical properties were determined, and the results are given in Table I.

Only very slight differences are noted in the yield of oils obtained from the plants grown on the different soils. The physical properties—color, odor, and taste—likewise show only slight variations. The specific gravity of the Arlington oils distilled during two seasons are lower than the South Dakota or Maryland oils. The rotation and refraction of the various oils differ considerably. The Arlington oils are slightly more soluble in 80 per cent alcohol than either of the other oils.

Comparing the chemical composition of oil distilled from the plants grown at Arlington with that from the South Dakota plants, it will be seen that the latter is richer in both esters and total menthol, while the ester content of the oil from Maryland plants is much greater than in either of the other oils.

From these results and from results obtained by other observers it may be inferred that light soils of either a sandy or loamy nature are more conducive to the production of esters and menthol in the oil than soils of heavy texture. In the case of the oils first compared it is probable that the climatic conditions during the growing period acted jointly with the soil conditions to bring about the difference in the oils. This relation, however, did not apply to the oils produced

<sup>1</sup> Henderson, H. J. An experiment in peppermint culture. *In Pharm. Jour.*, v. 87 (n. s., v. 33), no. 2493, p. 175-176. 1911.

<sup>2</sup> Umney, J. C. The effects of climate and soils on oils of peppermint. *In Pharm. Jour.*, v. 57 (s. 4, v. 3), no. 1362, p. 103-104. 1896.

<sup>3</sup> Charabot, Eugène, and Hébert, Alex. Influence de la nature de milieu extérieur sur l'état d'hydratation de la plante. *In Compt. Rend. Acad. Sci. [Paris]*, t. 136, no. 3, p. 160-163. 1903.

<sup>4</sup> Mossler, Gustave. Ueber den Einfluss verschiedener Kulturbedingungen auf das ätherische Oel von *Mentha piperita*. *In Pharm. Post*, Jahrg. 45, no. 1, p. 2-5. 1912.

from the plants grown at Glenn Dale, Md., and Arlington Farm, Va., since these are but a few miles apart and the general climatic conditions are practically the same in both localities.

TABLE I.—*Comparison of yields and physical composition of peppermint oils distilled from plants grown in several localities on various types of soils.*

| Locality and soil.                     | Yield of oil.         | Physical properties.                                                                  |                   |                        |                 |                                    | Chemical composition (per cent). |                                |          |        |
|----------------------------------------|-----------------------|---------------------------------------------------------------------------------------|-------------------|------------------------|-----------------|------------------------------------|----------------------------------|--------------------------------|----------|--------|
|                                        |                       | Color, odor, and taste.                                                               | Specific gravity. | Rotation, 50 mm. tube. | Re-fraction.    | Solubility in 80 per cent alcohol. | Free acid (as acetic).           | Free esters (menthyl acetate). | Menthol. |        |
|                                        |                       |                                                                                       |                   |                        |                 |                                    |                                  |                                | Free.    | Total. |
| Arlington Farm, Va. (heavy clay soil). | <i>P. ct.</i><br>0.12 | Pale yellow; agreeable menthol-like odor.                                             | <i>a</i> 0.9203   | — 10.6                 | <i>b</i> 1.4662 | <i>Volumes.</i><br><i>c</i> 1.2    | 0.21                             | 7.07                           | 46.5     | 51.7   |
| Do.....                                | .13                   | Pale yellow; mild, agreeable, minty odor; fatty, cooling, pungent taste.              | <i>a</i> . 9273   | — 5.7                  | <i>a</i> 1.4820 | <i>d</i> .6                        | .03                              | 11.67                          | 30.94    | 40.13  |
| Webster, S. Dak. (rich black loam).    | .10                   | Pale straw; pleasant flowery odor; pungent, minty, cooling taste.                     | <i>a</i> . 9280   | — 8                    | <i>b</i> 1.4715 | <i>d</i> 1.5                       | .05                              | 9.9                            | 46.48    | 54.28  |
| Glenn Dale, Md. (light sandy soil).    | .11                   | Pale yellow; agreeable aromatic, minty odor; bitter, slightly pungent, cooling taste. | <i>a</i> . 9403   | — 10                   | <i>c</i> 1.4735 | <i>d</i> .8                        | .75                              | 23.34                          | 35.89    | 54.28  |

*a* At 22° C.

*b* At 25° C.

*c* Turbid in 2 volumes or more.

*d* Turbid in 2½ volumes or more.

*e* At 23° C.

#### YIELD OF OIL FROM FRESH AND FROM DRY PLANTS AT VARIOUS STAGES OF GROWTH.

In order to study the effect of the time of harvest upon the yield and quality of peppermint oil and also the yield of oil as obtained from fresh and dry plants, experiments were conducted through a series of years, from 1908 to 1912, inclusive. The plants were harvested at three distinct stages of growth, namely, the budding, flowering, and fruiting stages, and the oil was distilled from both the fresh and the dried plants. A comparison was made of the various oils, the results being shown in Table II.

The yield of oils distilled from the fresh plants during the budding stage shows considerable variation, the yield in 1908 being especially high. During 1909, 1910, 1911, and 1912 considerably less variation is observed. Similar deviation during the flowering stage is to be noted, the yield in 1908 again being noticeably higher. More uniformity is apparent in the results obtained from the fruiting plant. In two of the seasons mentioned (1909 and 1911) the yield of oil during the fruiting stage was slightly in excess of that from either the budding or the flowering stage.

TABLE II.—*Yield of peppermint oil from the fresh and from the dry herb at various dates of distillation and stages of growth during five successive years.*

[All yields of oil calculated on the basis of fresh herb.]

| Description of material. | 1908     |               | 1909    |               | 1910     |               | 1911     |               | 1912     |               | Average.      |
|--------------------------|----------|---------------|---------|---------------|----------|---------------|----------|---------------|----------|---------------|---------------|
|                          | Date.    | Yield.        | Date.   | Yield.        | Date.    | Yield.        | Date.    | Yield.        | Date.    | Yield.        |               |
| Fresh herb:              |          | <i>P. ct.</i> |         | <i>P. ct.</i> |          | <i>P. ct.</i> |          | <i>P. ct.</i> |          | <i>P. ct.</i> | <i>P. ct.</i> |
| Budding...               | July 22  | 0.23          | July 14 | 0.13          | July 28  | 0.10          | July 17  | 0.12          | Aug. 5   | 0.09          | 0.134         |
| Flowering...             | Aug. 21  | .20           | July 24 | .12           | Aug. 16  | .13           | Aug. 17  | .09           | Sept. 21 | .12           | .132          |
| Fruiting...              | Sept. 25 | .10           | Aug. 10 | .17           | Sept. 16 | .10           | Sept. 18 | .13           | Oct. 5   | .07           | .114          |
| Dry herb:                |          |               |         |               |          |               |          |               |          |               |               |
| Budding.....             |          |               | July 19 | .080          | Aug. 5   | .070          | Aug. 15  | .050          |          |               | .066          |
| Flowering.....           |          |               | July 26 | .042          | Sept. 8  | .022          | Sept. 11 | .088          |          |               | .050          |
| Fruiting.....            |          |               | Aug. 19 | .052          | Sept. 27 | .048          | Oct. 2   | .040          |          |               | .046          |

The average percentage of moisture in the budding, flowering, and fruiting plants during the years 1909, 1910, and 1911 was 78.2, 74.2, and 69 per cent, respectively. The yield of oils obtained from the dried plants is lower in every case than from the fresh material. The general average yield is approximately 50 per cent lower than the yield obtained from the fresh herb at the same stage of growth. It appears, therefore, that prolonged drying of the herb before distillation is decidedly detrimental to the yield of oil obtainable.

In an experiment to determine what effect drying the plant has on the yield and constants of the oil, Todd<sup>1</sup> states that no loss of oil results when the plant is dried before distillation and that the color and solubility of the oil from the dried plants are superior to those from the fresh plants. The specific gravity, however, is slightly higher.

From the general averages during the three successive stages of growth of the plants, it appears that the yield of oil tends to decrease as the plant matures.

Information regarding the distribution of the oil in the plant was obtained by distilling separately the leaves and the tops of the plants at the three successive stages of growth and comparing these results with those recorded for the whole fresh herb. The results of these experiments are shown in Table III.

TABLE III.—*Comparison of yields of peppermint oil from the fresh leaves, from the tops, and from the whole fresh herb at different stages of growth during three successive years.*

| Parts of plant and stage of growth. | Yield of oil (per cent). |      |      |          |
|-------------------------------------|--------------------------|------|------|----------|
|                                     | 1909                     | 1910 | 1911 | Average. |
| Whole herb:                         |                          |      |      |          |
| Budding.....                        | 0.13                     | 0.10 | 0.12 | 0.116    |
| Flowering.....                      | .12                      | .13  | .09  | .113     |
| Fruiting.....                       | .17                      | .10  | .13  | .133     |
| Leaves:                             |                          |      |      |          |
| Budding.....                        | .20                      | .15  | .26  | .203     |
| Flowering.....                      | .27                      | .25  | .39  | .303     |
| Fruiting.....                       | .13                      | .08  | .15  | .120     |
| Tops:                               |                          |      |      |          |
| Budding.....                        | .20                      | .10  | .22  | .173     |
| Flowering.....                      | .16                      | .12  | .42  | .233     |
| Fruiting.....                       | .19                      | .08  | .19  | .153     |

<sup>1</sup> Todd, A. M. An experiment with peppermint plants. *In Amer. Drug.*, v. 15, no. 9, p. 161. 1886.

The results show that the yield of oil from the whole herb in the budding and flowering stages is consistently lower than that from the leaves and tops. In the fruiting stage, however, a slight deviation is observed. The yield of oil from the fresh herb is slightly higher in some instances than that from the leaves and tops. In practically all cases, however, the average yield from the leaves and tops during the three successive years is higher than the average from the whole herb at the three stages of growth.

From these results it is concluded that the largest portion of the oil in the peppermint plant is found in the leaves. The flowering tops contain slightly less than the leaves, and the stems are nearly devoid of oil. This latter fact possibly accounts for the lower percentage of oil in the whole plant as compared with that from the leaves and tops.

#### PHYSICAL AND CHEMICAL PROPERTIES OF PEPPERMINT OIL FROM FRESH AND FROM DRY PLANTS AT VARIOUS STAGES OF GROWTH.

In order to make possible a comparison of the properties of the various oils, a careful physical and chemical examination was made, and the results are presented in Table IV.

TABLE IV.—*Physical properties and chemical composition of peppermint oil distilled from the fresh and from the dry herb at various stages of growth during successive years.*

| Year and description of material. | Physical properties.                                                     |                   |                        |                 |                                    | Chemical composition (per cent). |                          |          |        |
|-----------------------------------|--------------------------------------------------------------------------|-------------------|------------------------|-----------------|------------------------------------|----------------------------------|--------------------------|----------|--------|
|                                   | Color, odor, and taste.                                                  | Specific gravity. | Rotation, 50 mm. tube. | Refraction.     | Solubility in 80 per cent alcohol. | Free acid (as acetic).           | Ester (menthyl acetate). | Menthol. |        |
|                                   |                                                                          |                   |                        |                 |                                    |                                  |                          | Free.    | Total. |
| 1908.                             |                                                                          |                   |                        |                 |                                    |                                  |                          |          |        |
| Fresh herb:                       |                                                                          |                   |                        |                 | Volumes.                           |                                  |                          |          |        |
| Budding.....                      | Pale yellow; pleasant menthol-like odor; cooling taste.                  | <i>a</i> 0.912    | — 15.8                 | <i>b</i> 1.4696 | <i>c</i> 1.5                       | 0                                | 9.55                     | 30.56    | 38.08  |
| Flowering.....                    | Pale yellowish green; pleasant esterlike odor; very pungent taste.       | <i>a</i> .924     | — 4.5                  | <i>b</i> 1.4728 | <i>c</i> 1.25                      | 0                                | 14.50                    | 23.97    | 35.39  |
| Fruiting.....                     | Yellowish green; pleasant minty odor; less pungent and cooling taste.    | <i>a</i> .918     | — 21                   | <i>b</i> 1.4681 | <i>c</i> 1.5                       | 0                                | 24.40                    | 34.33    | 53.55  |
| 1909.                             |                                                                          |                   |                        |                 |                                    |                                  |                          |          |        |
| Fresh herb:                       |                                                                          |                   |                        |                 |                                    |                                  |                          |          |        |
| Budding.....                      | Golden yellow; pleasant, faint odor; slightly pungent and cooling taste. | <i>d</i> .9158    | — 9.2                  | <i>e</i> 1.4738 | <i>f</i> 1                         | .10                              | 6.72                     | 30.1     | 35.39  |
| Flowering.....                    | Pale yellow; agreeable menthol-like odor; pungent, minty taste.          | <i>d</i> .9203    | — 10.6                 | <i>e</i> 1.4662 | <i>g</i> 1.2                       | .21                              | 7.07                     | 46.5     | 52.09  |
| Fruiting.....                     | Straw colored; very flowery odor; minty, pungent, taste.                 | <i>d</i> .9151    | — 8.3                  | <i>e</i> 1.4676 | <i>h</i> .8                        | .10                              | 12.37                    | 33.8     | 43.58  |

*a* At 23° C.

*b* At 24° C.

*c* Clear solution.

*d* At 22° C.

*e* At 25° C.

*f* Clear in excess.

*g* Turbid in 2 volumes or more.

*h* Turbid in 1½ volumes or more.

TABLE IV.—*Physical properties and chemical composition of peppermint oil distilled from the fresh and from the dry herb at various stages of growth during successive years—Continued.*

| Year and description of material. | Physical properties.                                                                 |                   |                        |             |                                    | Chemical composition (per cent). |                          |          |       |
|-----------------------------------|--------------------------------------------------------------------------------------|-------------------|------------------------|-------------|------------------------------------|----------------------------------|--------------------------|----------|-------|
|                                   | Color, odor, and taste.                                                              | Specific gravity. | Rotation, 50 mm. tube. | Refraction. | Solubility in 80 per cent alcohol. | Free acid (as acetic).           | Ester (menthyl acetate). | Menthol. |       |
|                                   |                                                                                      |                   |                        |             |                                    |                                  |                          | Free.    | Total |
| 1909.                             |                                                                                      |                   |                        |             |                                    |                                  |                          |          |       |
| Dry herb:                         |                                                                                      |                   |                        |             |                                    |                                  |                          |          |       |
| Budding.....                      | Greenish yellow; strong minty odor; pungent, minty taste.                            | a0.9337           | —13.1                  | b1.4695     | Volumes.<br>c 1.2                  | 0.17                             | 13.26                    | 36.07    | 46.38 |
| Flowering.....                    | Golden yellow; minty odor; pungent, minty, slightly fatty taste.                     | a.9163            | —13.5                  | b1.4695     | (d)                                | .16                              | 12.73                    | 37.37    | 47.44 |
| Fruiting.....                     | Yellow; unpleasant minty odor; strong, pungent, minty taste.                         | a.9344            | — 8.1                  | b1.4718     | e 1                                | .36                              | 17.68                    | 34.2     | 48.15 |
| 1910.                             |                                                                                      |                   |                        |             |                                    |                                  |                          |          |       |
| Fresh herb:                       |                                                                                      |                   |                        |             |                                    |                                  |                          |          |       |
| Budding.....                      | Dark golden; not fragrant, minty odor; slightly bitter, pungent, minty taste.        | f.9155            | —12.1                  | a1.4718     | g.7                                | .86                              | 16.62                    | 43.76    | 56.85 |
| Flowering.....                    | Pale yellow; mild, agreeable odor; fatty, strong, cooling, pungent taste.            | f.9273            | — 5.7                  | a1.4820     | e.6                                | .03                              | 11.67                    | 30.94    | 40.13 |
| Fruiting.....                     | Yellowish green; very fragrant flowery odor; very bitter and slightly pungent taste. | f.9250            | — 7                    | a1.4712     | h.8                                | 1.14                             | 20.86                    | 12.7     | 29.14 |
| Dry herb:                         |                                                                                      |                   |                        |             |                                    |                                  |                          |          |       |
| Budding.....                      | Light golden; herb-like, faint, minty odor; slightly bitter, pungent, minty taste.   | f.9167            | —11                    | a1.4724     | h.6                                | 1.20                             | 19.80                    | 39.41    | 55.01 |
| Flowering.....                    | Pale brown; herb-like odor; pungent and cooling taste.                               | .....             | .....                  | a1.4765     | .....                              | 3.20                             | 30.41                    | .....    | ..... |
| Fruiting.....                     | Golden yellow; fragrant, pleasant odor; very bitter and slightly pungent taste.      | f.9383            | — 3.3                  | a1.4713     | i.5                                | .56                              | 20.51                    | .....    | ..... |
| 1911.                             |                                                                                      |                   |                        |             |                                    |                                  |                          |          |       |
| Fresh herb:                       |                                                                                      |                   |                        |             |                                    |                                  |                          |          |       |
| Budding.....                      | .....                                                                                | .....             | .....                  | .....       | .....                              | .06                              | 12.02                    | 32.37    | 41.84 |
| Flowering.....                    | .....                                                                                | .....             | .....                  | .....       | .....                              | .20                              | 13.79                    | 34.11    | 44.97 |

a At 22° C.

b At 25° C.

c Turbid in excess.

d Turbid in 1 volume absolute alcohol; insoluble in 80 and 90 per cent alcohol in any proportion.

e Turbid in 2 volumes or more.

f At 24° C.

g Turbid in 4 volumes or more.

h Turbid in 1½ volumes or more.

i Turbid in 1¼ volumes or more.

Table IV shows that the oils distilled from the fresh herb collected at the three stages of growth show only slight differences in their general properties—color, odor, and taste. The oils from the flowering plants show a higher specific gravity and comparatively lower rotation than those from the budding and fruiting stages. With respect to their solubility in 80 per cent alcohol the several oils vary considerably.

From these physical properties it may be concluded that in several instances there are material differences in the composition of the oils, since the physical properties are greatly influenced by the constituents of the oils.

The color of the oils from the dry herb is uniformly darker than that of the oils from the fresh herb and the odor is distinctly less agreeable. The specific gravity is noticeably higher than in the oils from the fresh herb, but the variations in refraction, rotation, and solubility are less striking. Conspicuous among the oils from the dry herb is that from the flowering plants in 1909. This oil was insoluble in any proportion of either 80 or 90 per cent alcohol.

Table IV also shows that the percentage of free acids, calculated as acetic acid, in the various oils from the fresh plant varies considerably, probably as a result of the breaking down of the ester of the oil into free acid and menthol or of the inhibition of the esterification of the acid and menthol with the formation of menthyl acetate.

On comparing the percentage of ester or menthyl acetate in the oils from the fresh herb during the three stages of growth, it will be observed that the proportion of this constituent increases as the plant develops. The oil obtained at the fruiting stage is in every case richer in esters than the oil from the flowering or budding plants. The content of menthyl acetate of the oils varies in the budding plant during the several seasons from 6.72 to 16.62 per cent, in the flowering plant from 7.07 to 14.5 per cent, and in the fruiting plant from 12.37 to 20.86 per cent.

The free menthol content of the oils does not show the same tendency to increase as is noted in the case of the ester content. A certain relationship, however, seems to exist between the ester content and that of free menthol, since in many cases when the ester content is high the free menthol content is low, and vice versa. The oil distilled from the fruiting plant in 1910, in which year the percentage of esters was the highest, has the lowest percentage of free menthol, while the oil from the flowering plant in 1909, which contained a low percentage of esters, possessed the highest percentage of free menthol. This, however, is not true in every instance. That a relationship of this kind should exist between the ester content and the free menthol content is but natural, as the higher the percentage of menthol in combination in ester form the lower is the percentage of free menthol.

The percentage of total menthol represents the combined content of free menthol and the menthol held in combination in the form of the ester. Therefore, the oils with the highest content of total menthol are those which contain the highest content of free menthol and also combined menthol, or ester. This is strikingly shown in

the oil from the fruiting plant in 1908, which contains 53.55 per cent of total menthol, and in the oils from the flowering plant in 1909 and the budding plant in 1910, which contain, respectively, 52.09 and 56.85 per cent of total menthol.

In comparing the oils from the dry herb with those from the fresh herb it will readily be observed that the content of free acetic acid is uniformly higher in the oil from the dry plants. It is interesting to note that in practically every instance the ester content is also higher than in the oils from the fresh plants. It would appear, therefore, that during the drying process there occurs a chemical change which is favorable to esterification. Notwithstanding the higher content of esters, the odor of the oils was decidedly inferior. This is attributed to the presence of certain volatile substances formed during drying, which distilled over with the oils and imparted an objectionable herby odor to them.

The free menthol and the total menthol content of the various oils from both the dry and the fresh plants appears to be similarly affected, as the percentage of both free and total menthol is uniformly high. It is, however, doubtful whether the increased content of ester and menthol in the dry plants would warrant the drying of the plants before distillation, since, as previously noted, a considerable percentage of oil is lost when the plants are dried.

The distillation of peppermint oil from plants in a fresh condition at different periods of growth up to the time of flowering has been reported by Charabot,<sup>1</sup> who finds that the density and rotation diminish during vegetation. The plant is richest in menthol at the appearance of vegetation and lowest in the combined menthol, or esters. As the growth of the plant progresses, the percentage of the combined menthol, or esters, increases.

A study was made of the physical and chemical properties of the oils distilled separately from the fresh leaves and flowering tops, and for comparison the results are presented with those obtained for the oils from the whole fresh herb, as shown in Table V.

The general characters of the oils from the leaves and tops at the successive stages of growth are very similar to those of the oils from the entire herb. The specific gravity and rotation of the oils from the leaves and the tops are higher in nearly every case than the same constants of the oils from the entire herb. The refractive indexes are variable, while the solubility in 80 per cent alcohol is less in most cases than that of the oils from the whole herb. These constant differences in specific gravity, rotation, and solubility apparently denote a difference in the composition of the oils from the leaves and tops as compared with that from the whole herb.

<sup>1</sup> Charabot, Eugène. Recherches sur les migrations et les métamorphoses des composés terpéniques dans la menthe poivrée. *In* Bul. Soc. Chim. Paris, s. 3, t. 23, p. 466-474. 1900.

TABLE V.—Comparison of the physical properties and the chemical composition of peppermint oils distilled from the fresh leaves, from the tops, and from the entire fresh herb at the various stages of growth.

| Year and description of material. | Physical properties.                                                                 |                   |                        |                 |                                    | Chemical composition (per cent). |                          |          |        |
|-----------------------------------|--------------------------------------------------------------------------------------|-------------------|------------------------|-----------------|------------------------------------|----------------------------------|--------------------------|----------|--------|
|                                   | Color, odor, and taste.                                                              | Specific gravity. | Rotation, 50 mm. tube. | Refraction.     | Solubility in 80 per cent alcohol. | Free acid (as acetic).           | Ester (menthyl acetate). | Menthol. |        |
|                                   |                                                                                      |                   |                        |                 |                                    |                                  |                          | Free.    | Total. |
| 1909.                             |                                                                                      |                   |                        |                 |                                    |                                  |                          |          |        |
| Budding stage:                    |                                                                                      |                   |                        |                 |                                    |                                  |                          |          |        |
| Herb.....                         | Golden yellow; pleasant, faint odor; slightly pungent, cooling taste.                | <i>a</i> 0.9158   | — 9.2                  | <i>b</i> 1.4738 | <i>Volumes.</i><br><i>c</i> 1      | 0.10                             | 6.72                     | 30.1     | 35.39  |
| Leaves.....                       | Straw colored; fragrant but faint minty odor; fatty, pungent, slightly bitter taste. | <i>a</i> .9143    | —12.1                  | <i>b</i> 1.4713 | <i>d</i> 1.4                       | .014                             | 9.9                      | 37.7     | 45.67  |
| Tops.....                         | Straw colored; very fragrant minty odor; fatty, bitter, pungent taste.               | <i>a</i> .9151    | —12                    | <i>b</i> 1.4718 | <i>e</i> 1                         | .116                             | 8.84                     | 31.8     | 38.76  |
| Flowering stage:                  |                                                                                      |                   |                        |                 |                                    |                                  |                          |          |        |
| Herb.....                         | Pale yellow; agreeable menthol-like odor; pungent, minty taste.                      | <i>a</i> .9203    | —10.6                  | <i>b</i> 1.4662 | <i>f</i> 1.2                       | .21                              | 7.07                     | 46.5     | 52.09  |
| Leaves.....                       | Straw colored; pleasant, minty odor; pungent taste.                                  | <i>a</i> .9339    | —11                    | <i>b</i> 1.4706 | <i>f</i> 1.2                       | .52                              | 17.3                     | 41.7     | 55.38  |
| Tops.....                         | Straw colored; flowery odor; pungent taste.                                          | <i>a</i> .9370    | —10.3                  | <i>b</i> 1.4732 | <i>f</i> 1                         | .48                              | 15.9                     | 37.75    | 50.29  |
| Fruiting stage:                   |                                                                                      |                   |                        |                 |                                    |                                  |                          |          |        |
| Herb.....                         | Straw colored; very flowery odor; minty, pungent taste.                              | <i>a</i> .9151    | — 8.3                  | <i>b</i> 1.4676 | <i>e</i> .8                        | .10                              | 12.37                    | 33.8     | 43.58  |
| Leaves.....                       | Golden yellow; pleasant, minty odor; minty, pungent taste.                           | <i>a</i> .9373    | — 9.6                  | <i>b</i> 1.4730 | <i>d</i> 1.5                       | .32                              | 19.8                     | 37.58    | 53.18  |
| Tops.....                         | Light straw colored; pleasant, flowery odor; minty, slightly pungent taste.          | <i>a</i> .9223    | — 9.5                  | <i>b</i> 1.4723 | <i>e</i> 1                         | .16                              | 15.56                    | 34.8     | 47.08  |
| 1910.                             |                                                                                      |                   |                        |                 |                                    |                                  |                          |          |        |
| Budding stage:                    |                                                                                      |                   |                        |                 |                                    |                                  |                          |          |        |
| Herb.....                         | Dark golden; not fragrant, minty odor; slightly bitter, pungent, minty taste.        | <i>g</i> .9155    | —12.1                  | <i>a</i> 1.4718 | <i>h</i> .7                        | .86                              | 16.62                    | 43.76    | 56.85  |
| Leaves.....                       | Golden yellow; faint, not especially minty odor; slightly bitter, pungent taste.     | <i>g</i> .9156    | —13.1                  | <i>a</i> 1.4710 | <i>h</i> .6                        | .90                              | 19.80                    | 18.79    | 34.39  |
| Tops.....                         | Golden yellow; agreeable, minty odor; strong, pungent, bitter, cooling taste.        | .....             | .....                  | .....           | .....                              | .13                              | 13.44                    | 29.19    | 39.78  |
| Flowering stage:                  |                                                                                      |                   |                        |                 |                                    |                                  |                          |          |        |
| Herb.....                         | Pale yellow; mild, agreeable odor; fatty, strong, cooling, pungent taste             | <i>g</i> .9273    | — 5.7                  | <i>a</i> 1.4820 | <i>d</i> .66                       | .03                              | 11.67                    | 30.94    | 40.13  |

*a* At 22° C.*b* At 25° C.*c* Clear in excess.*d* Turbid in 3 volumes or more.*e* Turbid in 1½ volumes or more.*f* Turbid in 2 volumes or more.*g* At 24° C.*h* Turbid in 4 volumes or more.

TABLE V.—*Comparison of the physical properties and the chemical composition of peppermint oils distilled from the fresh leaves, from the tops, and from the entire fresh herb at the various stages of growth—Continued.*

| Year and description of material. | Physical properties.                                                               |                   |                        |             |                                    | Chemical composition (per cent). |                          |          |        |
|-----------------------------------|------------------------------------------------------------------------------------|-------------------|------------------------|-------------|------------------------------------|----------------------------------|--------------------------|----------|--------|
|                                   | Color, odor, and taste.                                                            | Specific gravity. | Rotation, 50 mm. tube. | Refraction. | Solubility in 80 per cent alcohol. | Free acid (as acetic).           | Ester (menthyl acetate). | Menthol. |        |
|                                   |                                                                                    |                   |                        |             |                                    |                                  |                          | Free.    | Total. |
| 1910.                             |                                                                                    |                   |                        |             |                                    |                                  |                          |          |        |
| Flowering stage—Continued.        |                                                                                    |                   |                        |             |                                    |                                  |                          |          |        |
| Leaves.....                       | Pale yellow; strong minty odor; slightly fatty, bitter, pungent, cooling taste.    | a 0.9231          | —9.4                   | b 1.4753    | Volumes.<br>c 0.7                  | 1.90                             | 22.27                    | 37.83    | 55.38  |
| Tops.....                         | Pale yellow; fragrant, agreeable odor; fatty, bitter, pungent, cooling taste.      | a. 9242           | —6.5                   | b 1.4780    | c. 8                               | 1.43                             | 22.98                    | 48.60    | 66.71  |
| Fruiting stage:                   |                                                                                    |                   |                        |             |                                    |                                  |                          |          |        |
| Herb.....                         | Yellowish green; very fragrant flowery odor; very bitter, slightly pungent taste.  | a. 9250           | —7                     | b 1.4712    | d. 8                               | 1.14                             | 20.86                    | 12.7     | 29.14  |
| Leaves.....                       | Golden yellow; strong minty odor; bitter, cooling, slightly pungent taste.         | .....             | .....                  | b 1.4720    | .....                              | 4.80                             | 43.40                    | .....    | .....  |
| Tops.....                         | Greenish yellow; agreeable, flowery odor; bitter, slightly pungent, cooling taste. | .....             | .....                  | b 1.4738    | .....                              | 3.30                             | 28.29                    | .....    | .....  |

a At 24° C.  
b At 22° C.

c Turbid in 1½ volumes or more.  
d Turbid in 2 volumes or more.

During all the stages of growth the content of free acetic acid is consistently higher in the oils from the leaves and tops than in the oils from the whole herb. High acidity is usually accompanied by a correspondingly high percentage of menthyl acetate. This is clearly demonstrated by the oils from the leaves and tops at the flowering and fruiting stages in 1910, when the percentage of acid varied from 1.14 to 4.8 and the menthyl acetate from 20.86 to 43.4 per cent.

The ester content of the oils from both leaves and tops increases as the plant matures, the fruiting stage producing the oils richest in this constituent. The percentage of esters in the oils from the leaves and tops is higher in nearly every instance than in the oil from the whole fresh herb. It is very probable, therefore, that the esterification of the acetic acid and menthol takes place most readily in these parts of the plant. The formation of esters is no doubt less in the stems, which accounts for the lower ester content of the oil distilled from the whole plant.

The percentage of free menthol in the leaves and tops during the various stages does not seem to bear any constant relationship to the ester content or to the free menthol content of the oil from the whole plant. The total menthol of leaves and tops, however, is higher in nearly all cases than that of the fresh herb.

It would seem from the foregoing results that the formation of the principal constituents of peppermint oil, namely, menthyl acetate and menthol, takes place chiefly in the leaves and tops of the plants, beginning at an early stage of growth and increasing as the plant matures.

#### EFFECT OF LIGHT AND SHADE.

In a study of the effect of soil and climatic conditions upon the constituents of plants the factor of light is considered important by certain investigators.

Géneau<sup>1</sup> states that strong light has a favorable effect upon the decomposition of carbon dioxid and the elimination of water vapor in plants. Berthelot<sup>2</sup> has observed that plants grown in the shade contain noticeably greater proportions of water than plants grown in the light.

Charabot and Hébert<sup>3</sup> have investigated the peppermint plant and find that darkness considerably reduces the percentage of volatile oil in the plant. It is also stated by these investigators<sup>4</sup> that plants kept in the shade consume the terpene constituents of the oil.

For the purpose of ascertaining the effect of shade on the yield and composition of peppermint oil, cultural experiments were conducted through two successive years upon a small plat of peppermint grown under a slat shade, whereby the light intensity was reduced about 50 per cent, and upon a similar plat grown in the open to serve as a check. The plants were harvested during the flowering stage and distilled in the fresh condition.

The physical and chemical properties of the respective oils were determined and for comparison are presented in Table VI.

It will be observed that in 1910 the yield of oil was the same from both the shade-grown and the unshaded plants, while in 1911 the yield from the shade-grown plants was slightly in excess of that from the unshaded plants. These results are at variance with those of earlier investigators with respect to the formation of oil in shade-grown plants. The plants described were, however, grown in only partial shade, which may have been the optimum condition for the formation of the oil in 1911.

<sup>1</sup> Géneau de Lamarlière, Léon. Recherches physiologiques sur les feuilles développées à l'ombre et au soleil. *In* Rev. Gén. Bot., t. 4, p. 494. 1892.

<sup>2</sup> Berthelot. Sur la marche générale de la végétation; plante développée à l'ombre et au soleil. *In* Compt. Rend. Acad. Sci. [Paris], t. 128, no. 3, p. 139-144. 1899.

<sup>3</sup> Charabot, Eugène, and Hébert, Alex. Formation des composés terpéniques dans les organes chlorophylliens. *In* Bul. Soc. Chim. Paris, s. 3, t. 31, p. 402-409. 1904.

<sup>4</sup> Charabot, Eugène, and Hébert, Alex. Consommation des matières odorantes chez la plante étiolée. *In* Bul. Soc. Chim. Paris, s. 3, t. 33, p. 580-585. 1905.

TABLE VI.—*Comparison of the yields, the physical properties, and the chemical composition of peppermint oils distilled from shade-grown and from unshaded plants.*

| Items of comparison.                        | 1910                                          |                                   | 1911                                   |                                          |
|---------------------------------------------|-----------------------------------------------|-----------------------------------|----------------------------------------|------------------------------------------|
|                                             | Shade-grown plants.                           | Unshaded plants.                  | Shade-grown plants.                    | Unshaded plants.                         |
| Yields and physical properties:             |                                               |                                   |                                        |                                          |
| Yield, per cent.                            | 0.13.....                                     | 0.13.....                         | 0.15.....                              | 0.9.                                     |
| Color.....                                  | Pale yellow.....                              | Pale yellow.....                  | Pale golden yellow.                    | Dark golden yellow.                      |
| Odor.....                                   | Very mild, minty..                            | Mild, agreeable, minty.           | Mild, agreeable, minty.                | Strong minty, characteristic.            |
| Taste.....                                  | Fatty, cooling, slightly bitter, and pungent. | Fatty, strong, cooling, pungent.  | Strongly bitter, pungent, minty.       | Bitter, pungent, strong, cooling.        |
| Specific gravity (at 23° C.).               | 0.9343.....                                   | 0.9273.....                       | 0.9192.....                            | 0.9233.                                  |
| Rotation, in 50 mm. tube, degrees.          | -2.24.....                                    | -5.7.....                         | -3.4.....                              | -7.3.                                    |
| Refraction.....                             | 1.4724, at 22° C.....                         | 1.4820 at 22° C.....              | 1.4708, at 23° C.....                  | 1.4722, at 23° C.                        |
| Solubility in 80 per cent alcohol, volumes. | 0.7: turbid in 1½ volumes or more.            | 0.6: turbid in 2 volumes or more. | 0.7: very turbid in 2 volumes or more. | 0.75: very turbid in 1½ volumes or more. |
| Chemical composition (per cent):            |                                               |                                   |                                        |                                          |
| Free acids (calculated as acetic acid).     | 0.....                                        | 0.03.....                         | 0.07.....                              | 0.20.                                    |
| Menthylacetate.                             | 10.96.....                                    | 11.67.....                        | 13.08.....                             | 13.79.                                   |
| Free menthol.....                           | 24.43.....                                    | 30.94.....                        | 31.20.....                             | 34.11.                                   |
| Total menthol...                            | 33.07.....                                    | 40.13.....                        | 41.50.....                             | 44.97.                                   |

Compared with the oils from unshaded plants, the oils obtained from the shade-grown plants in 1910 showed a slight increase in the specific gravity and in 1911 but very little difference. The rotation and refraction of the oils from the shade-grown plants were lower in both years than in the oils from the unshaded plants. In solubility but little difference was noticed. In general appearance, however, the oils from the shade-grown plants were somewhat lighter in color and showed a slight difference in odor and taste. The marked difference in rotation of the two oils during both seasons is especially significant and denotes differences in composition.

The free acidity of the oils from the shade-grown plants is seen to be lower in both seasons than that of the oil from the unshaded plants, as is also the content of menthyl acetate, free menthol, and total menthol. The results indicate that both esterification and the formation of menthol are retarded by the absence of light, possibly because photosynthetic activities are greater in the presence of light.

#### EFFECT OF FROST ACTION.

From the considerations presented in the foregoing paragraphs it seems probable that in the peppermint plant the formation of certain compounds and the breaking down of others is dependent upon a number of factors, some of which stimulate while others retard, or possibly inhibit, the reactions taking place in the plant tissues. The changes which take place in a volatile oil as a result of the action of

these factors upon the process of synthesis in the plant are usually confined to the least stable constituents.

The process of esterification is common to most aromatic plants and is more or less affected by the conditions of temperature, light, and moisture under which the plant grows. Among these factors the effect of frost action upon the formation of aromatic compounds in plants has perhaps received least attention, and whether it promotes or retards the development of the odorous compounds in the oil is not yet definitely known. Charabot,<sup>1</sup> in an investigation on the relation of esters to the chlorophyll in plants, states that the effect of reduced temperature on the formation of esters is opposite to that of either intense light or a dry atmosphere. Schimmel & Co.,<sup>2</sup> in a report on the oil distilled from frozen leaves of the bergamot mint (*Mentha citrata* Ehrh.) grown in Florida, state that the yield of oil did not differ materially from that of the unfrozen plant, but that the physical and chemical properties disclosed marked variations. The most striking difference was the large increase in ester content (linalyl acetate) of the oil from the frozen leaves, the oil from the unfrozen plants containing only one-third as much ester.

In the course of some studies on the peppermint plant made at Arlington Farm during the seasons of 1910 and 1911, advantage was taken of the second crop, which came on late in the autumn, to collect some data regarding the effect of frost action upon the new growth of plants. These plants were from 12 to 18 inches tall, in an early although fairly vigorous stage of growth, and bore an abundance of leaves. After having been exposed to several heavy frosts the plants were distilled and the oils subjected to a thorough examination. As a basis for comparison, plants unaffected by frost were distilled each year. In 1910, plants from the first crop in an early stage of growth, and consisting mainly of leaves with no buds, were distilled on July 25. In 1911, plants from the second crop, which had made a short growth after the harvesting of the first crop, were distilled on October 30. These plants consisted of leaves with a few buds. Table VII gives the results of the examinations.

An inspection of Table VII shows that the yield of oil from both the frozen and unfrozen plants is practically the same, although it is considerably lower than that from fully grown plants, the yield from which is approximately double that here recorded. The oil from the frozen plants had the more agreeable and flowery odor, which may be attributed to the very high content of esters of menthol. The cooling sensation resulting when the oil from the frozen plants was applied to the tongue was noticeably greater than from that of

<sup>1</sup> Charabot, Eugène. Sur le rôle de la fonction chlorophyllienne dans l'évolution des composés terpéniques. *In* Compt. Rend. Acad. Sci. [Paris], t. 132, no. 3, p. 159-161. 1901.

<sup>2</sup> Schimmel & Co. (Fritzsche Brothers). Semiannual Report, p. 14. Miltitz, London, New York, Nov., 1908.

the oil from the unfrozen plants, possibly because of the greater proportion of menthol in the first-mentioned oil.

The physical constants reveal marked differences in the rotation and some differences in the solubility in 80 per cent alcohol. The higher rotation of the oil from the frozen plants is attributable to the presence of a greater quantity of the strongly levogyrate compounds, menthol and menthyl acetate.

TABLE VII.—*Comparison of the yields, the physical properties, and the chemical composition of peppermint oils distilled from frozen and from unfrozen plants.*

| Items of comparison.                       | 1910                                      |                                    | 1911                                       |                                    |
|--------------------------------------------|-------------------------------------------|------------------------------------|--------------------------------------------|------------------------------------|
|                                            | Frozen plants.                            | Unfrozen plants.                   | Frozen plants.                             | Unfrozen plants.                   |
| Physical properties:                       |                                           |                                    |                                            |                                    |
| Yield, per cent.....                       | 0.065.....                                | 0.07.....                          | 0.11.....                                  | 0.077.                             |
| Color.....                                 | Pale straw.....                           | Pale yellowish green.              | Dark golden yellow.                        | Pale golden yellow.                |
| Odor.....                                  | Mild, flowery, characteristic minty.      | Strongly minty.....                | Flowery, not strongly minty.               | Strongly minty.                    |
| Taste.....                                 | Bitter, pungent, and exceedingly cooling. | Pungent, slightly bitter, cooling. | Bitter, pungent, and cooling.              | Bitter, slightly pungent, cooling. |
| Specific gravity.                          | 0.9107, at 24° C.....                     | 0.9142, at 24° C.....              | 0.9155, at 23° C.....                      | 0.9180, at 23° C.                  |
| Rotation, in 50 mm. tube, degrees.         | -11.5.....                                | -8.43.....                         | -16.5.....                                 | -11.7.                             |
| Refraction.....                            | 1.4592, at 25° C.....                     | 1.4689, at 22° C.....              | 1.4580, at 23° C.....                      | 1.4621, at 23° C.                  |
| Solubility in 80 per cent alcohol, volume. | 0.4; clear in excess.                     | 0.6; turbid in 1½ volumes or more. | 0.6; faintly turbid in 3½ volumes or more. | 0.5; turbid in 2½ volumes or more. |
| Chemical composition (per cent):           |                                           |                                    |                                            |                                    |
| Free acid (calculated as acetic acid).     | 0.09.....                                 | 0.086.....                         | 0.09.....                                  | 0.12.                              |
| Menthyl acetate.                           | 24.3.....                                 | 8.8.....                           | 40.66.....                                 | 31.82.                             |
| Free menthol.....                          | 40.7.....                                 | 31.8.....                          | 28.93.....                                 | 9.99.                              |
| Total menthol.....                         | 67.8.....                                 | 38.7.....                          | 60.97.....                                 | 35.06.                             |

The oils from the frozen plants, being readily soluble, remain more or less clear when an excess of 80 per cent alcohol is added, whereas the oils from the unfrozen plants become turbid in one and one-half volumes or more of alcohol. In the oils from the frozen plants, therefore, a higher content of the easily soluble menthol and menthyl acetate, and possibly a lower content of the more or less insoluble terpenes, may be expected.

In discussing the chemical constants of the oils it is noted that the quantity of free acids is nearly the same for all the oils. The ester content of the oils from the frozen plants, when calculated as menthyl acetate, was 34.3 in 1910 and 40.66 per cent in 1911, which is much higher than the ester content of the oils from the unfrozen plants. During both seasons the percentage of free menthol in the oils from the frozen plants exceeded that in the oils from the unfrozen plants. The total menthol content was also much higher in the oils from the frozen plants than in that from the unfrozen plants.

If, as in the ordinary esterification reactions, the formation of esters in the plant takes place by the action of the organic acids upon

the free alcohols with the elimination of water, it is possible that the end result of the reaction would be intensified provided there was some means whereby the water could be rendered incapable of reversing the reaction.

According to Charabot,<sup>1</sup> who has investigated ester formation in the peppermint plant, the esterification takes place in the green parts of the plant as a result of "chlorophyllic evaporation" of water from the leaves, while the process of oxidation of menthol to menthone takes place in the floral parts of the plant. It has been further demonstrated by Charabot and Hébert<sup>2</sup> that conditions which tend to increase the elimination of water favor the esterification in the plant. The addition of salt (NaCl) to the soil was shown to be followed by an increase in the percentage of esters in the oil. The probability that esterification in a plant is aided by the presence of an enzym with a reversible action has also been suggested.<sup>3</sup>

Whatever the explanation of the wide variation in the oils may be, it seems evident that the effect of frost upon the peppermint plant tends to intensify esterification.

If esterification is favored by a reduced water content in the plant due to increased evaporation, as appears from the work of Charabot and Hébert, freezing may bring about the same result, either by checking the absorption of water by the plant or by removing from the sphere of action the water already contained in it. Also esterification may have continued after freezing by the aid of catalytic agents, the resultant water being frozen as formed, thus diminishing the chances of a reverse action.

Since the percentage of free menthol was slightly higher in the oil from the frozen plant, and as the menthol in the plant has been shown to be capable of oxidation to menthone, possibly another effect of freezing is to lessen oxidation in the plant tissues.

### CONCLUSIONS.

Conditions of soil and climate are influential factors in the formation of oil and its constituents in the peppermint plant. Light sandy or loamy soils appear to be most favorable for the production of an oil of high quality.

The yield of oil distilled from fresh plants apparently decreases as the plant matures. Drying the plant before distillation results in a considerable loss of oil. The largest proportion of oil is found in the leaves and flowering tops.

<sup>1</sup> Charabot, Eugène. Recherches sur les migrations et les métamorphoses des composés terpéniques dans la menthe poivrée. *In* Bul. Soc. Chim. Paris, s. 3, t. 23, p. 466-474. 1900.

<sup>2</sup> Charabot, Eugène, and Hébert, Alex. Chemical investigations on the vegetation of perfume-yielding plants. *In* Bul. Sci. and Indus., Roure-Bertrand Fils, s. 1, no. 5, p. 24. 1902.

<sup>3</sup> Charabot, Eugène, and Hébert, Alex. Recherches sur le mécanisme de l'éthérification chez les plantes. *In* Bul. Sci. et Indus. Roure-Bertrand Fils, s. 1, no. 4, p. 3-16. 1901.

The percentage of esters in the oils increases as the plants approach maturity. The menthol content of the oil bears a close relationship to the ester content. The free acidity and ester content of the oil distilled from dry plants is considerably higher than in the oil from fresh plants. The drying of the plants causes changes favorable to esterification, while the percentage of free and total menthol in oils distilled from dried plants is also uniformly high.

The formation of esters and menthol takes place most readily in the leaves and tops of the plant, the metabolic processes showing increased activity as the plant matures.

The effect of shade upon the peppermint plant is to decrease esterification and the formation of menthol, and is due possibly to the lessened activity of the elimination of water by the plant.

The action of frost noticeably increases esterification and the formation of menthol.

---

---

ADDITIONAL COPIES

OF THIS PUBLICATION MAY BE PROCURED FROM  
THE SUPERINTENDENT OF DOCUMENTS

GOVERNMENT PRINTING OFFICE

WASHINGTON, D. C.

AT

5 CENTS PER COPY

▽



NATIONAL AGRICULTURAL LIBRARY



1022734408